DOCKET NO.: ORT-1518 Application No.: 09/975,650 Office Acti n Dated: July 25, 2003

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Original) A method for measuring ligand binding to a vanilloid receptor comprising the steps of:

- (a) forming in an aqueous solution having a pH in the range of about 8.0 7.5 to about 10.0 a liquid composition comprising a test compound, a labeled ligand, and a human VR1 at least a ligand-interacting portion of a vanilloid receptor protein;
- (b) incubating the solution for a time sufficient to permit the test compound and labeled ligand to contact the vanilloid receptor;
- (c) measuring the amount of labeled ligand bound to the vanilloid receptor protein; and
- (d) determining if the test compound binds to the vanilloid receptor protein bound to the receptor by observing a reduction in the amount of expected labeled ligand bound to the vanilloid receptor protein, as compared with an equivalent aqueous solution comprising the labeled ligand and the vanilloid receptor protein, but not the test compound.
 - 2. Canceled
 - 3. Canceled.
 - 4. Canceled.
- 5. (Original) The method of claim 1 wherein the pH is in the range of about pH 8.1 to about 9.1.
 - 6. (Original) The method of claim 1 wherein the labeled ligand is a radiolabeled ligand.

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- 7. (Original) The method of claim 6 wherein the radiolabeled ligand is tritiated resiniferatoxin.
- 8. (Currently amended) The method of claim 1 additionally comprising the steps after the incubating step of:

removing unbound labeled ligand from the solution; and isolating the receptor protein. [[;]]

- 9. (Currently amended) The method of claim 1 wherein the aqueous buffer further comprises a divalent cation selected from the group consisting of:
 - (a) magnesium at a final concentration of between about 1 to about 5mM; and
 - (b) calcium at a final concentration of about 0.1mM to about 2 mM.
- 10. (Original) The method of claim 9 wherein the magnesium concentration is about 2mM.
- 11. (Original) The method of claim 9 wherein the calcium concentration is about 0.8 mM.
- 12. Canceled. (Original) The method of claim 9 wherein the vanilloid receptor is a human vanilloid receptor.
- 13. (Currently amended) The method of claim <u>8</u> 1 wherein the removing step comprises adding a sufficient <u>quantity</u> quality of alpha 1 acid glycoprotein to the aqueous solution to adsorb unbound labeled ligand.
 - 14. (Original) The method of claim 1 wherein the steps are performed in order.

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15. (Currently amended) The method of claim <u>8</u> 9 wherein the <u>removing</u> isolating step is performed before the isolating <u>removing</u> step.

- 16. (Currently amended) A method to measure ligand binding to a vanilloid receptor comprising the steps, in order
- (a) combining in an aqueous solution having a pH in the range of about 8.0 7.5 to about 10.0, a test compound, a labeled ligand, and a human VR1 vanilloid receptor protein, said protein being associated with a portion of a cell membrane;
- (b) incubating the solution for sufficient time for the test compound and ligand to contact the vanilloid receptor <u>protein</u>;
- (c) adding a sufficient quantity of alpha 1 acid glycoprotein to the solution to adsorb unbound labeled ligand;
 - (d) isolating the membrane from the aqueous solution;
- (e) measuring the amount of labeled ligand bound to the <u>vanilloid receptor</u> protein in the membrane; and
- (f) determining if the test compound <u>binds to the vanilloid receptor protein bound to</u> the receptor by observing a reduction in the amount of <u>expected</u> labeled ligand <u>bound to the</u> vanilloid receptor protein, as compared with an equivalent aqueous solution comprising the labeled ligand and the vanilloid receptor protein, but not the test compound.
- 17. (Currently amended) A method to measure compound binding to a vanilloid receptor comprising the steps, in order, of:
- (a) combining in an aqueous solution having a pH of about 8.6, a test compound, a radiolabeled resiniferatoxin, and a human <u>VR1</u> vanilloid receptor-1 (VR1) protein, said protein being a portion of a cell membrane;
- (b) incubating the solution for sufficient time for the test compound and the resiniferatoxin to contact the vanilloid receptor protein;

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- (c) adding a sufficient quantity of alpha 1 acid glycoprotein to the solution to adsorb unbound resiniferatoxin;
 - (d) isolating the membrane from the aqueous solution;
- (e) measuring the amount of resiniferatoxin bound to the <u>vanilloid receptor</u> protein in the membrane; and
- (f) determining if the test compound <u>binds the vanilloid receptor protein</u> bound to the receptor by observing a reduction in the amount of expected resiniferatoxin <u>bound to the vanilloid receptor protein</u>, as compared with an equivalent aqueous solution comprising the resiniferatoxin and the vanilloid receptor protein, but not the test compound.
- 18. (Original) The method of claim 17 wherein the buffer also contains a divalent cation selected from the group consisting of:
 - (a) magnesium at a final concentration of about 2mM; and
 - (b) calcium at a final concentration of about 0.8mM.